

Key Characters for the Microscopical Identification of *Cylicocycclus nassatus* and *Cylicocycclus ashworthi* (Nematoda: Cyathostominae) of the Horse, *Equus caballus*

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ABSTRACT: Current efforts to develop improved control methods for nematode parasites of horses have been hampered by difficulties in identifying some nematodes of the genus *Cylicocycclus*. Structural characteristics of several species of *Cylicocycclus* parasitic in domesticated horses, *Equus caballus*, are described that facilitate the identification. Key microscopical characteristics are described for *C. nassatus* and *C. ashworthi* including characteristics useful for separating them from the similar species *C. triramosus*, *C. leptostomus*, and *C. radiatus*. *Cylicocycclus nassatus* is characterized by a cuticular shelf on the inner surface of the buccal capsule, a dorsal gutter that is as long as 50% of the buccal capsule depth, 20 elements in the external leaf crown (ELC) that each have a sharp tip that broadens quickly to form a parallel-sided leaf, and lateral papillae that produce a tall, narrow cuticular extension of the mouth collar. Both *C. nassatus* and *C. ashworthi* have a short, rounded dorsal bursal lobe in which the proximal branch (of 3 on each side) overlaps 75–80% of the middle branch and a female tail that is slightly longer than the vulva to anus distance. *Cylicocycclus ashworthi* can be distinguished from *C. nassatus* by the absence of a shelf on the inner surface of the buccal capsule, by its much shorter dorsal gutter that is wider than long, and by its 25–29 ELC elements that taper gradually throughout their length and lateral papillae that produce a short, broad extension in the cuticle of the mouth collar. Three other species of the genus, *C. leptostomus*, *C. radiatus*, and *C. triramosus*, all have males with elongate dorsal bursal lobes in which the proximal branch overlaps less than 50% of the middle branch and females with a tail that is much shorter (*C. triramosus*) or slightly shorter (*C. leptostomus* and *C. radiatus*) than the vulva to anus distance. *Cylicocycclus leptostomus* can be distinguished by its small buccal capsule, *C. radiatus* by its large buccal capsule without a dorsal gutter, and *C. triramosus* by its extremely short dorsal gutter and ventral and dorsal notches in the mouth collar. Our study of paratypes of *C. ashworthi* and *C. matumurai* resulted in synonymizing the latter with the former.

Strongyloid nematodes of the subfamily Cyathostominae cause significant morbidity in domesticated horses. More than 40 species occur, sometimes in large numbers, in the large intestine, and resistance to antiparasitic drugs is common among these nematodes. Considerable research is underway around the world to develop alternate or improved control strategies. This research requires accurate identification of the nematode species. Modern identification manuals and classifications of the Cyathostominae exist (Lichtenfels, 1975, 1980; Hartwich, 1986), but problems in identifying several species of the genus *Cylicocycclus* Ihle, 1922, persist. *Cylicocycclus nassatus* (Looss, 1900) Chaves, 1930, is one of the most common nematodes in the ventral colon of horses. Over the years several similar species have been described: *C. triramosus* (Yorke and Macfie, 1920) Chaves, 1930;

C. ashworthi (LeRoux, 1924) McIntosh, 1933; and *C. matumurai* (Yamaguti, 1942) Lichtenfels, 1975, have been recognized in the recent literature. In addition, a subspecies, *C. nassatus parvum* (Yorke and Macfie, 1918), has been listed by most recent authors (Lichtenfels, 1975; Hartwich, 1986) as a synonym of *C. nassatus*. The present report of a study of specimens (including types of most species; Table 1) concludes that, of the preceding species, only *C. nassatus* and *C. ashworthi* occur commonly in domesticated horses and provides information necessary for their identification based on light microscopy of cephalic characteristics. Information is also presented that facilitates the identification of *C. radiatus* (Looss, 1900) Chaves, 1930, and *C. leptostomus* (Kotlan, 1920) Chaves, 1930, and the distinction of these species from *C. nassatus* and *C. ashworthi*. A redescription of *C. triramosus*,

Table 1. Number and sex of type and voucher specimens studied.

Species	USNPC No.*	Number and sex studied	Type	Collector
<i>Cylicocyclus ashworthi</i>	149†	5 males, 5 females	Paratypes	P. L. LeRoux
	31544‡	1 male	—	W. L. Threlkeld
	33344‡	1 male, 1 female	—	Van Volkenberg
	33345	1 male, 1 female	—	Van Volkenberg
	33346	1 male	—	Van Volkenberg
	50860‡	3 females	—	M. Tubangui
	69887§	3 males, 6 females	—	B. J. Torbert
	70387§	3 males, 1 female	—	C. Sommer
	79151§	3 males, 4 females	—	S. L. Eduardo
	82941§	10 males, 10 females	—	O. Slocombe
	83890§	4 males	—	G. M. Dvojnos
	83405§	1 male	—	M. Ito
	85068§	1 male	—	J. Monrad
	85076§	1 female	—	J. Monrad
	85080	1 male	—	J. Monrad
	85092	12 males, 3 females	—	J. Monrad
	85101	1 male	—	J. Monrad
	85120	1 female	—	J. Monrad
	85191	2 males, 1 female	—	J. Monrad
	85226	1 female	—	J. Monrad
	85280	1 female	—	J. Monrad
	86419	1 male, 1 female	—	J. R. Georgi
	86420	1 male, 1 female	—	J. R. Georgi
	86421	1 male, 1 female	—	J. R. Georgi
	86422	3 males, 4 females	—	J. R. Georgi
<i>Cylicocyclus leptostomus</i>	58489	4 males, 5 females	—	A. O. Foster
	85137	1 female	—	J. Monrad
	85178	1 female	—	J. Monrad
	85190	4 females	—	J. Monrad
	85281	2 males	—	J. R. Georgi
	86423	1 male, 1 female	—	J. R. Georgi
<i>Cylicocyclus matumurai</i>	86424	1 male, 2 females	—	J. R. Georgi
	22565	1 male, 2 females	Paratypes	S. Yamaguti
<i>Cylicocyclus nassatus</i>	9602	14 males, 41 females	Paratypes	A. Looss
	31544‡	3 males	—	W. L. Threlkeld
	32422‡	4 males, 15 females	—	H. C. Clark
	33342	1 male	—	Van Volkenberg
	33343‡	3 females	—	Van Volkenberg
	33344‡	3 males, 1 female	—	Van Volkenberg
	50860‡	4 males, 3 females	—	M. Tubangui
	58396	5 males, 5 females	—	A. O. Foster
	83405	4 males, 2 females	—	M. Ito
	83405	6 males, 3 females	—	M. Ito
	85179	3 males, 1 female	—	J. Monrad
	85189	9 males, 15 females	—	J. Monrad
	85225	1 female	—	J. Monrad
	86425	2 males, 2 females	—	J. R. Georgi
	86426	5 males, 5 females	—	J. R. Georgi
	86427	2 males, 2 females	—	J. R. Georgi
	86428	3 males, 3 females	—	J. R. Georgi
	86429	3 males, 3 females	—	J. R. Georgi
	86430	23 males, 16 females	—	J. R. Georgi
<i>Cylicocyclus triramosus</i>	78995	2 males, 2 females	—	R. C. Krecsek

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† British Museum Natural History Collection Number, London.

 ‡ Redetermined: originally identified as *C. nassatus parvum*.

 § Redetermined: originally identified as *C. triramosus*.

|| Meguro Parasitology Museum Collection Number, Tokyo.

believed to be a species encountered only in South African zebras, will be provided separately (V.A. Kharchenko 1996, pers. comm.), but key identifying features are described herein. The status of *C. nassatus parvum* remains uncertain (species indeterminata) with type specimens unavailable.

Materials and Methods

Specimens studied are listed in Table 1. Scientific names follow those used by Lichtenfels (1975).

Specimens were studied in temporary wet mounts, cleared in phenol-alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) with the aid of interference contrast light microscopy.

Measurements listed in Table 2 are from previous reports and in micrometers unless indicated otherwise.

Results

The identification of species of *Cylicocyclus*, like other Cyathostominae, can be made solely on the basis of cephalic characters (Lichtenfels, 1975). The results presented here are intended to provide a description of characters useful for identifying *C. nassatus* (Figs. 1–8) and *C. ashworthi* (Figs. 9–16). In addition, characters useful for distinguishing these species from *C. leptostomus*, *C. radiatus*, and *C. triramosus* are described (Table 2). Characteristics of the dorsal ray (Figs. 8, 16) of the male copulatory bursa and the ratio of female tail length to vulva to anus distance and vagina length (Figs. 7, 15) provide additional characters useful for separating these species.

The most distinctive characteristic of *C. nassatus* (Figs. 1–8) is the cuticular shelf-like projection that rings the internal walls of the buccal capsule at about midway in its depth (Figs. 1, 2). No other species of the genus *Cylicocyclus* has such a feature, and it can be seen with $\times 400$ magnification even in most poor specimens. Other supplemental identifying features of the cephalic region include a dorsal gutter that is one-half as long as the depth of the buccal capsule (Figs. 2, 6); lateral cephalic papillae that protrude through the mouth collar sufficiently to produce a pointed, steeply sided projection of the mouth collar (Fig. 5); submedian cephalic papillae with elongate banana-shaped tips (Fig. 3); elements of the external leaf crown (ELC) that have tips that broaden quickly in the anterior one-fourth of their length and then taper more gradually toward their base (Fig. 3); and elements of the internal leaf crown (ILC) (Figs.

1, 2) that are shorter than the thickness of the ring at the base of the buccal capsule.

Cylicocyclus ashworthi (Figs. 9–16) is similar to *C. nassatus* grossly, but it lacks a cuticular buccal capsule shelf and can be separated from its co-inhabitant of the ventral colon by differences in numerous cephalic characteristics. Characteristic features of *C. ashworthi*, in addition to lacking the cuticular buccal capsule shelf, include a dorsal gutter that is less than one-third as long as the depth of the buccal capsule (Fig. 14); lateral cephalic papillae that protrude through the mouth collar to form a broad, rounded projection of the mouth collar (Fig. 13); submedian cephalic papillae with tapering, pointed tips (Fig. 14); ELC elements with uniformly, gradually tapering tips for more than one-half of their length (Fig. 11); and ILC elements that are longer (Figs. 9, 10) than the thickness of the ring at the base of the buccal capsule.

A study of paratypes of *C. ashworthi* found no differences between them and other specimens identified and described herein as *C. ashworthi*. Many lots of specimens previously identified as *C. triramosus* from horses, *E. caballus*, have been redetermined as *C. ashworthi* (Table 1).

En face counts of ELC elements found specimens collected in Japan fell into 2 groups: 1 with 20 and another with 26–28 ELC elements. Specimens with 20 ELC elements also had the characteristics already described for *C. nassatus*. Specimens with 26–28 ELC elements had the characteristics described herein for *C. ashworthi*.

A single male and 2 female paratypes of *Cylicocyclus matumauri* were found to have all the characteristics of *C. ashworthi*.

The dorsal bursal rays of both *C. nassatus* and *C. ashworthi* have 3 branches on each side (Figs. 8, 16). The middle and distal branches of the dorsal ray of *C. ashworthi* frequently have various, variable accessory branches (Fig. 16). However, about 30–50% of paratype males of *C. nassatus* also have such accessory branches, and this is not a reliable character for identifying the species. Accessory branches were also present on the branches of dorsal rays of *C. triramosus* and *C. leptostomus*.

Discussion

Although the most distinctive characteristic of the species *C. nassatus* is the cuticular shelf in the lining of the buccal capsule, this structure

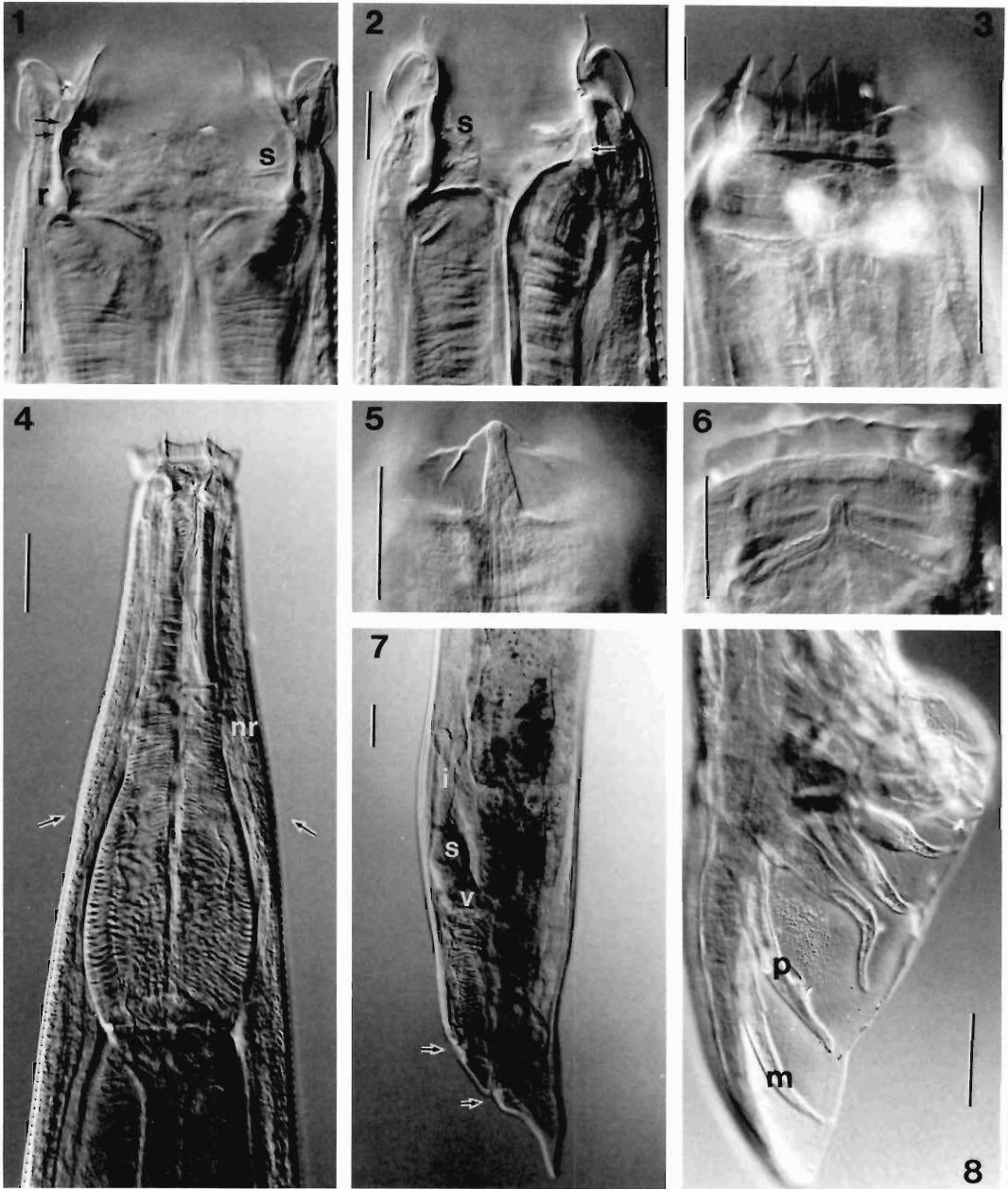
Table 2. Morphometrics of key characteristics of *Cylicocycylus* spp.*

	Host: Distribution: Prevalence: Site:	<i>C. nassatus</i> <i>E. caballus</i> Cosmopolitan Common Ventral colon	<i>C. ashworthi</i> <i>E. caballus</i> Cosmopolitan Common Ventral colon	<i>C. tritamosus</i> <i>E. burchelli antiquorum</i> South Africa Common Ventral colon	<i>C. leptostomus</i> <i>E. caballus</i> Cosmopolitan Common Cecum, colon	<i>C. radianus</i> <i>E. caballus</i> Cosmopolitan Rare Colon
Body length ♂/♀ (in mm)		8–10 [†] 9–14 [†]	7.4–8.6 ² 8.5–9.5 [†]	12.3 ³ 12.3 ³	6 ⁴ 7–8 ⁴	11 ¹ 13–14 ¹
Buccal capsule width/depth		10–135 [†] 35–47 [†]	65–100 [†] 19–23	90–110 ³ 38 ³	36 ⁴ 18 ⁴	112 ⁴ 52 ⁴
Dorsal gutter shape/% of depth of buccal capsule		Taller than wide >50%	Wider than tall 35%	Taller than wide 16–22%‡	Taller than wide 20%	Absent ¹
Number ELC elements		19–20 ⁸	25–29 ⁸	30 ³	24–26 ⁸	26 ¹
Distal tip of proximal branch of dorsal ray		Overlaps 80% of middle branch	Overlaps 75% of middle branch	Overlaps <50% of middle branch	Overlaps none of middle branch	Overlaps 20% of middle branch
Dorsal bursal ray shape		Short, rounded	Short, rounded	Slightly elongated	Elongate	Elongate
Vagina length		514–806 [†]	311–349 [†]	712–851 [‡]	300–400 ⁴	600–750 ⁴
Vulva to anus		139–188 [†]	79–131 [†]	290–360 ⁴	80–90 ⁴	250–280 ⁴
Female tail length		180–243 [†]	112–146 [†]	160–200 ⁴	64–70 ⁴	200–250 ⁴

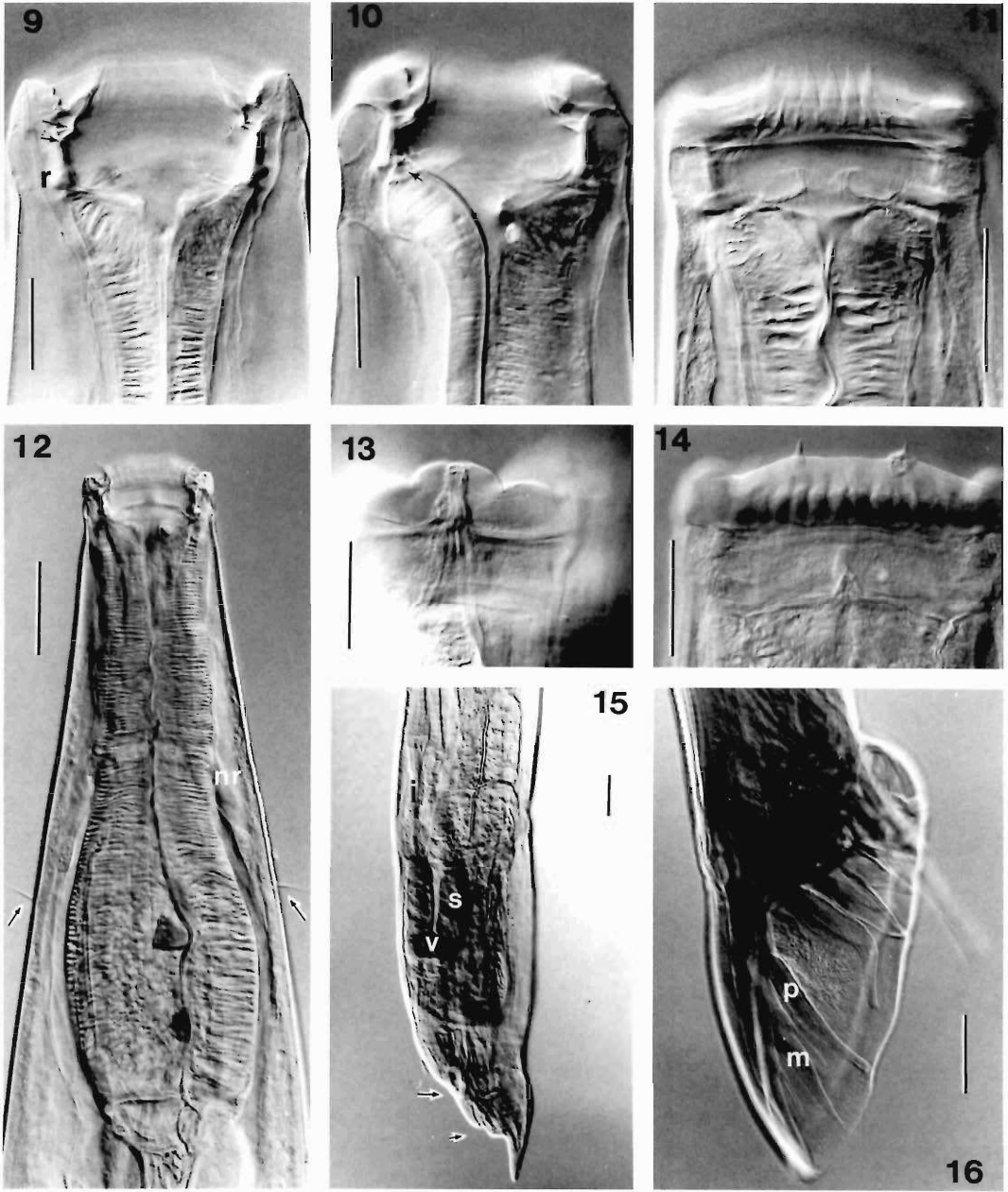
* ¹Braide and Georgi; ¹Looss, 1900; ²LeRoux, 1924; ³Yorke and Macfie, 1920; ⁴Theiler, 1923.

[†] Measurements of 5 female paratypes, original.

[‡] Measurements of 2 female specimens.



Figures 1-8. *Cylicocycylus nassatus*, photomicrographs. Scale bars, Figs. 1-3, 5, 6, = 50 μ m; Figs. 4, 7, 8 = 100 μ m. 1. Buccal capsule, dorsoventral view, showing cuticular shelf on inner lining (s), tall, lateral papillae, ring-like thickening at base of buccal capsule (r) and short ILC element (between arrows). 2. Buccal capsule, lateral view, showing cuticular shelf (s) and dorsal gutter (arrow). 3. Mouth collar, dorsoventral view, showing several elements of ELC and tip of submedian papilla. 4. Esophageal region, dorsoventral view, showing position of nerve ring (nr), cervical papillae (arrows) and shape of esophagus. 5. Lateral papilla protruding through mouth collar. 6. Dorsal gutter. 7. Female tail showing anus and vulva (arrows) and ovejectors, including ventricle (v), sphincters (s) and infundibulae (i). 8. Male copulatory bursa showing position and shape of bursal rays, especially the proximal (p) and medial (m) branches of the dorsal ray.



Figures 9–16. *Cyllococyclus ashworthi*, photomicrographs. Scale bars, Figs. 9–11, 13, 14, = 50 μm; Figs. 12, 15, 16 = 100 μm. 9 Buccal capsule, dorsoventral view, showing tall, lateral papillae, ring-like thickening at base of buccal capsule (r) and short ILC element (between arrows). 10. Buccal capsule, lateral view, showing dorsal gutter (arrow). 11. Mouth collar, dorsoventral view, showing several elements of ELC. 12. Esophageal region, dorsoventral view, showing position of nerve ring (nr), cervical papillae (arrows) and shape of esophagus. 13. Lateral papilla protruding through mouth collar. 14. Dorsal gutter and submedian papilla. 15. Female tail showing anus and vulva (arrows) and ovejectors, including ventibule (v), sphincters (s) and infundibulae (i). 16. Male copulatory bursa showing position and shape of bursal rays, especially the proximal (p) and medial (m) branches of the dorsal ray.

was not emphasized by Looss (1900, 1902) in his description of the species. Looss (1902) did describe the cuticular shelf but believed it to be a variable structure. We suspected that perhaps the reason Looss (1902) believed it to be variable might be the possible inclusion of specimens later described as *C. nassatus parvum* (which lack the cuticular buccal shelf) within his series of specimens of *C. nassatus*. Looss (1902) mentioned that his type series included smaller perfectly mature specimens, "not more than 8 millimeters in the male and 9 millimeters in the female," measurements typical of the smaller *C. nassatus parvum* later described by Yorke and Macfie (1918). However, we examined all of the 55 paratypes of *C. nassatus* available to us without finding a single specimen that fit the description of *C. nassatus parvum*. Every paratype of *C. nassatus* fit the characterization of this species presented herein. In the description of *C. nassatus parvum*, the authors did not mention or figure a cuticular shelf in the lining of the buccal capsule. Unfortunately, the types of *C. nassatus parvum* were not found either at the British Museum or at the International Institute of Parasitology, St. Albans, England. Among voucher specimens identified as *C. nassatus parvum* (Table 1), we found specimens that fit the characterization of either *C. nassatus* or *C. ashworthi* presented in this report. In the absence of type specimens of *C. nassatus parvum*, we must consider this subspecies to be unidentifiable, or a species indeterminata.

We now propose to recognize the name *C. ashworthi* for the common species from domesticated horses, previously reported either as *C. ashworthi* or as *C. triramosus* by numerous authors worldwide. Braide and Georgi (1974), the only North American workers to correctly identify this species, reported 25–29 ELC elements in *C. ashworthi*. Lichtenfels (1975) listed *C. ashworthi* as a synonym of *C. nassatus* following several earlier workers and *C. triramosus* as a species reported in Puerto Rico but not known to occur in North America because no specimens were available for study. Hartwich (1986) synonymized *C. ashworthi* with *C. triramosus* after studying syntypes of *C. ashworthi*. Other authors have reported *C. ashworthi* from horses in Brazil (Lanfredi and Honer, 1984) and *C. triramosus* from horses in the United States (Torbert et al., 1986), the Ukraine (Dvojnos and Kharchenko, 1990), and the Philippine Islands

(Antiporda and Eduardo, 1990) and from Burchell's zebra (Scialdo-Krecek, 1983). However, one of us (J.R.L.) has studied paratypes of *C. ashworthi* and specimens collected by Scialdo-Krecek (1983) from a South African zebra, *Equus burchelli*, the type host of *C. triramosus*, and have concluded (with V. A. Kharchenko 1996, pers. comm.) that the latter is a distinct species that appears, as suggested by Theiler (1923), to occur only in South African zebras. *Cylicocyclus triramosus* is distinguished by an extremely short dorsal gutter and ventral and dorsal notches in the mouth collar. It will be re-described separately (Kharchenko et al., 1997). We now can confirm that only the *C. triramosus* from South African zebras should be considered to represent that species. All available lots of "*C. triramosus*" from *E. caballus* examined in this study (Table 1) have been redetermined as *C. ashworthi*.

It appears that the morphological characteristics of *C. ashworthi* and *C. matumurai* are so similar that they should be considered synonyms. Hartwich (1986) did not study *C. matumurai*, but followed Lichtenfels (1975) in recognizing this species. However, Lichtenfels (1975) did not study *C. matumurai* but recognized it as a species of the genus that had not been reported in North America. After studying a single male and 2 female paratypes of *C. matumurai*, we believe it is a synonym of *C. ashworthi*. Yamaguti (1942) presented an excellent en face drawing that shows 26 ELC elements and other characteristics of *C. ashworthi* described herein are also present in *C. matumurai*.

Other species of *Cylicocyclus* of horses that might be confused with *C. ashworthi* or *C. nassatus* include the rare species *C. radiatus* and the common species *C. leptostomus*. Of these species, *C. radiatus* is the only one lacking a dorsal gutter and *C. leptostomus* has a small buccal capsule (Table 2). Males of both *C. radiatus* and *C. leptostomus* have elongate dorsal bursal rays in which there is a considerable distance between the proximal and middle branches of the dorsal ray so that only the tip of the proximal branch overlaps the origin of the middle branch. In contrast, in *C. ashworthi* and *C. nassatus* males, the dorsal bursal ray is short and rounded and most of the length of the proximal branch overlaps the origin of the middle branch (Table 2). Females of *C. leptostomus* and *C. radiatus* can be separated from those of *C. nas-*

satus and *C. ashworthi* because their tails are slightly shorter than their vulva to anus distance (Table 2). In many older females, however, tail structure is distorted and cephalic characters are more useful for species determinations.

Acknowledgments

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